Assessment of the added clinical value of next generation sequencing of the 16S-23S rRNA region in a clinical setting

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BACKGROUND

Accurate identification of bacterial species in clinical samples facilitates optimal antibiotic treatment of the individual patient. The use of next generation sequencing (NGS) methods in diagnostic medical microbiology is increasing as these methods overcome most limitations of culture and 16S rDNA Sanger sequencing (16S Sanger). Here we present a retrospective proof of principle study to reveal the added clinical value of NGS of the 16S-23S rRNA region (16S-23S NGS) when applied to a variety of clinical samples.

MATERIALS AND METHODS

- 25 samples from 19 patients suspected for complex infections were subjected to culture and 16S Sanger sequencing, both using routine standardized methods, and to 16S-23S NGS (Sabat *et al.*, 2017)
- The added clinical relevance of 16S-23S rDNA NGS was assessed using an algorithm, including criteria defining the added clinical value, by clinical microbiologists (Figure 1)

CONCLUSIONS

- This proof of principle study demonstrates that 16S-23S rDNA NGS:
  - is of added clinical value, enabling identification of bacterial species in complex samples
  - has the potential to be integrated into the routine diagnostic workflow
- However, this approach needs further clinical evaluation in multidisciplinary teams

RESULTS

- Culture positive: 8 samples (32%)
- 16S Sanger positive: 11 samples (44%)
  5 samples mixed sequences
- 16S-23S NGS positive: 22 samples (88%)
  a single bacterial species: 9 samples
  polymicrobial communities: 13 samples

- The added clinical value of 16S-23S NGS was evident in clinical materials of 7 of 19 patients (37%)
  - Brain abscess (n=2)
  - Joint tissue (n=1)
  - Blood vessel tissue (n=1)
  - Joint punctate (n=1)
  - Pleural fluid (n=1)
  - Blood culture (n=1)

Sample	Material	Culture	16S PCR (Ct-value)	16S Sanger*	16S-23S rDNA NGS Identification	Bacterial fraction (%)	Eukaryote fraction (%)	Background fraction (%)	Clinical assessment
1	Brain abscess	*Porphyromonas asaccharolytica* *Porphyromonas somerae* *Parvimonas micra*	17	No ID	*Fusobacterium* spp. *Porphyromonas* spp.	99,5 0,2	0,3	0	A
4	Blood culture	Unidentified gram positive rods	16	No ID	*Actinotignum* spp.	100	0	0	B
7	Joint tissue	Negative	Negative	No ID	*Rothia mucilaginosa* *Corynebacterium* spp. *Cutibacterium acnes*	0,2 1,9 0,9	22,4	74,1	B
8	Joint punctate	Negative	Negative	No ID	*Capnocytophaga canimorsus*	99,1	0,9	0	B
10	Blood vessel tissue	Negative	38	No ID	*Cutibacterium acnes* *Staphylococcus epidermidis* *Anaerococcus* spp.	6,7 7,2 2,5	23,6	60	B
20	Brain abscess	Negative	26	No ID	*Dialister pneumosintes* *Parvimonas micra* and 18 additional identifications	12,3 5,5	0,1	54,1	B
25	Pleural fluid	*Fusobacterium nucleatum*	22	No ID	*Prevotella pleuritidis* *Fusobacterium nucleatum* *Actinomyces meyeri*	77,5 21,1 1,4	0	0	A

* No ID: No identification due to mixed sequences

x Number of patients with score A, B, C1, C2 or D

Assessment of added clinical relevance of 16S-23S rDNA NGS

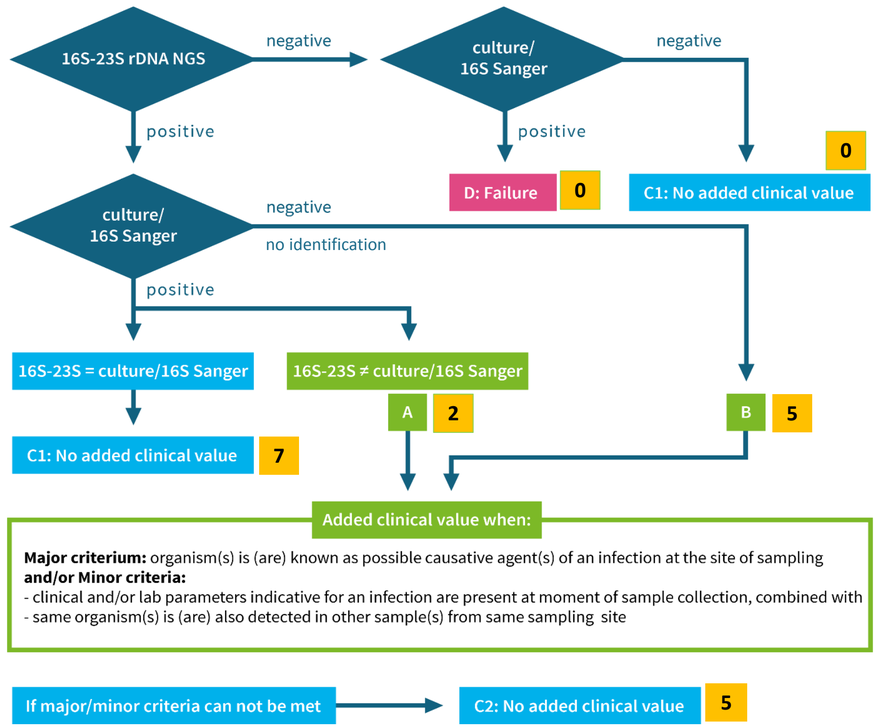

Figure 1: Flowdiagram to assess the added clinical relevance of 16S-23S rDNA NGS for patients.